Supplementary Figures and Tables

Supplementary Table S1: Collection sites for populations of *Drosophila melanogaster* from the east coast of Australia

Population	Latitude (°S)	Longitude(°E)
Townsville	19.26	146.79
Rockhampton	23.15	150.72
Brisbane	27.61	153.30
Ballina	28.87	153.44
Coffs Harbour	30.23	153.15
Port Macquarie	30.93	152.90
Wollongong	34.34	150.91
Narooma	36.25	150.14
Gosford	33.31	151.20
Bermagui	36.40	150.06
Melbourne	37.99	145.27

Table S2: Multilevel model examining the effects of sex and haplotype (A1, B1) and sub-haplotype nested within haplotype on the ability to tolerate the extreme heat challenge. Sex, haplotype and sub-haplotype were modelled as fixed effects. Duplicate nested within population was modelled as a random effect alongside Trial ID.

Fixed effects	**Chisq**	**df**	**Pr (> Chisq)**
(Intercept)	1276.14	1	<0.001
Haplotype	6.04	1	0.0139
Sex	109.32	1	<0.001
Haplotype × Sex	24.73	1	<0.001
Sub-haplotype[Haplotype]	0.98	3	0.804
Sub-haplotype[Haplotype] × Sex	25.04	3	<0.001
Random effects	**St.Dev**		
Duplicate[Population]	1.109		
Population	0		
Trial ID	0.857		

Table S3: Multilevel model examining the effects of sex and haplotype (A1, B1) and sub-haplotype nested within haplotype on the ability to tolerate the extreme cold challenge. Sex haplotype and sub-haplotype were modelled as fixed effects. Duplicate nested within population was modelled as a random effect alongside scorer (person recording the data).

Fixed effects	**Chisq**	**df**	**Pr(>Chisq)**
(Intercept)	739.78	1	< 0.001
Haplotype	34.32	1	< 0.001
Sex	21.06	1	< 0.001
Sub-haplotype[Haplotype]	3.85	3	0.075
Haplotype × Sex	3.07	1	0.080
Sub-haplotype[Haplotype] × Sex	7.50	3	0.0574
Random effects	**St.Dev**		
Duplicate[Population]	0.255		
Population	1.56		
Scorer	0.585		

Table S4: General linear model examining the effects of haplotype (A1, B1) and gene identity on mitochondrial gene expression. mtDNA copy number was added as a fixed covariate in the analysis.

	Sum Sq	df	F value	Pr(>F)
(Intercept)	3.32	1	77.051	< 0.001
Haplotype	0.03	1	0.715	0.399
Gene	109.14	4	632.664	< 0.001
Copy N.	0.11	1	2.752	0.1
Haplotype × Gene	1.41	4	8.196	< 0.001
Residual	4.27	99		

Table S5: Linear model examining the effects of haplotype (A1, B1) on mtDNA copy number.

	Sum Sq	df	F value	Pr(>F)
(Intercept)	336.8	1	640.319	< 0.001
Haplotype	1.68	1	3.187	0.082
Residual	19.99	38		

Table S6A: 2 × 2 contingency table for AT and GC substitutions between the A1 and B1 haplotypes. A Fishers exact test was used to test for departures from the expectation of equal numbers in the two classes. The P value = 0.0028, indicating that the proportion of nucleotide sites with A-T is higher in the B1 than the A1 haplotype.

	A-T	**C-G**
North	3	12
South	12	3

Table S6B: 2 × 2 contingency table for preferred and unpreferred codons between the A1 and B1 haplotypes. A Fishers exact test was used to test for departures from the expectation of equal numbers in the two classes. The P value = 0.0012, indicating the proportion of preferred codons is higher in the south haplogroup than the north haplogroup.

	Preferred	**Unpreferred**
North	2	11
South	11	2

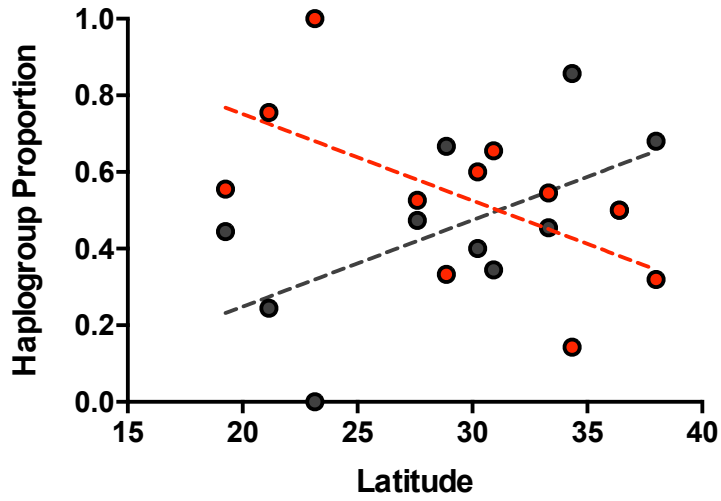

Figure S1: Frequency of each haplogroup with latitude. The A haplogroup is denoted in red, and the B haplogroup in grey (A & B: $R^2 = 0.3679$, $p \leq 0.05$).

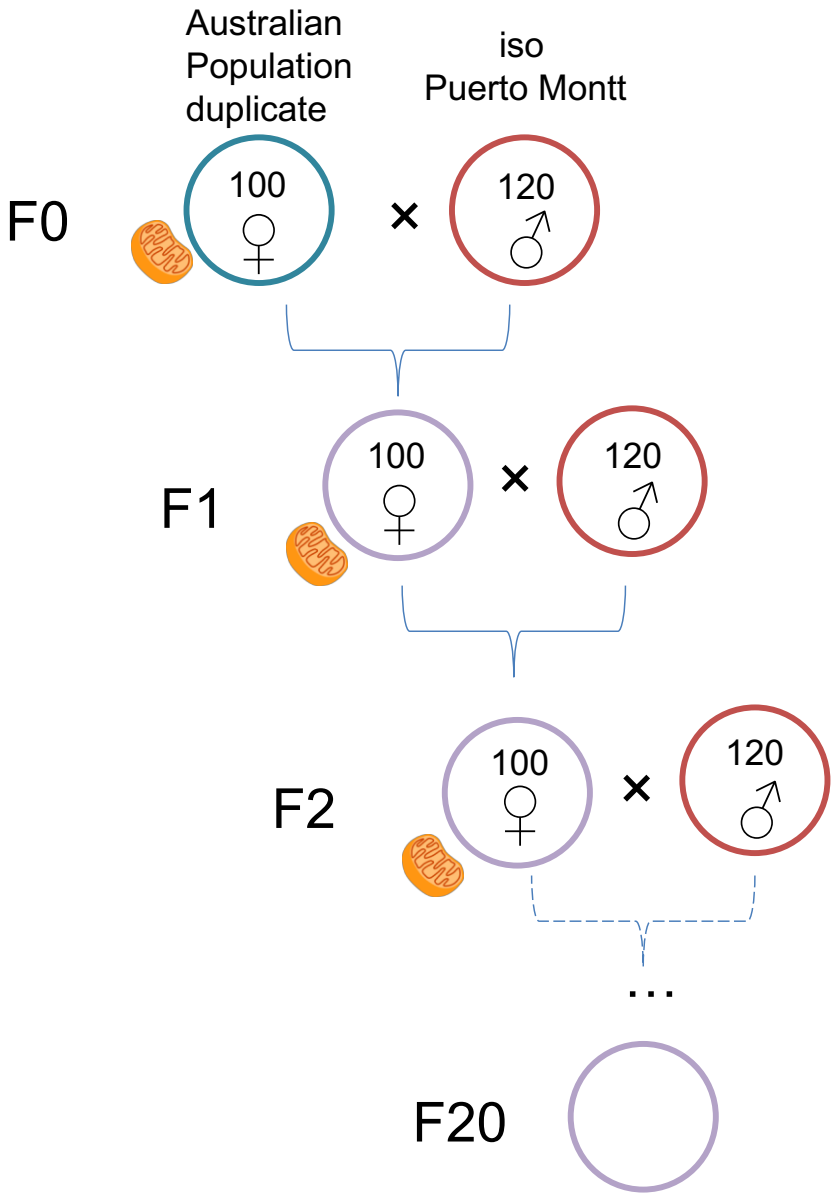

Figure S2A: Schematic of experimental setup (part 1). Eleven populations were collected from along the Australian east coast, and two independent duplicates from each location were kept as mass-bred populations (11 populations × 2 duplicates = 22 experimental units). Females (n = 100) of each population duplicate were then backcrossed to males (n = 120) of an isogenic line from Puerto Montt (PUE) for 20 sequential generations. This figure represents the introgression regime for one experimental unit (one duplicate within a mass-bred population).

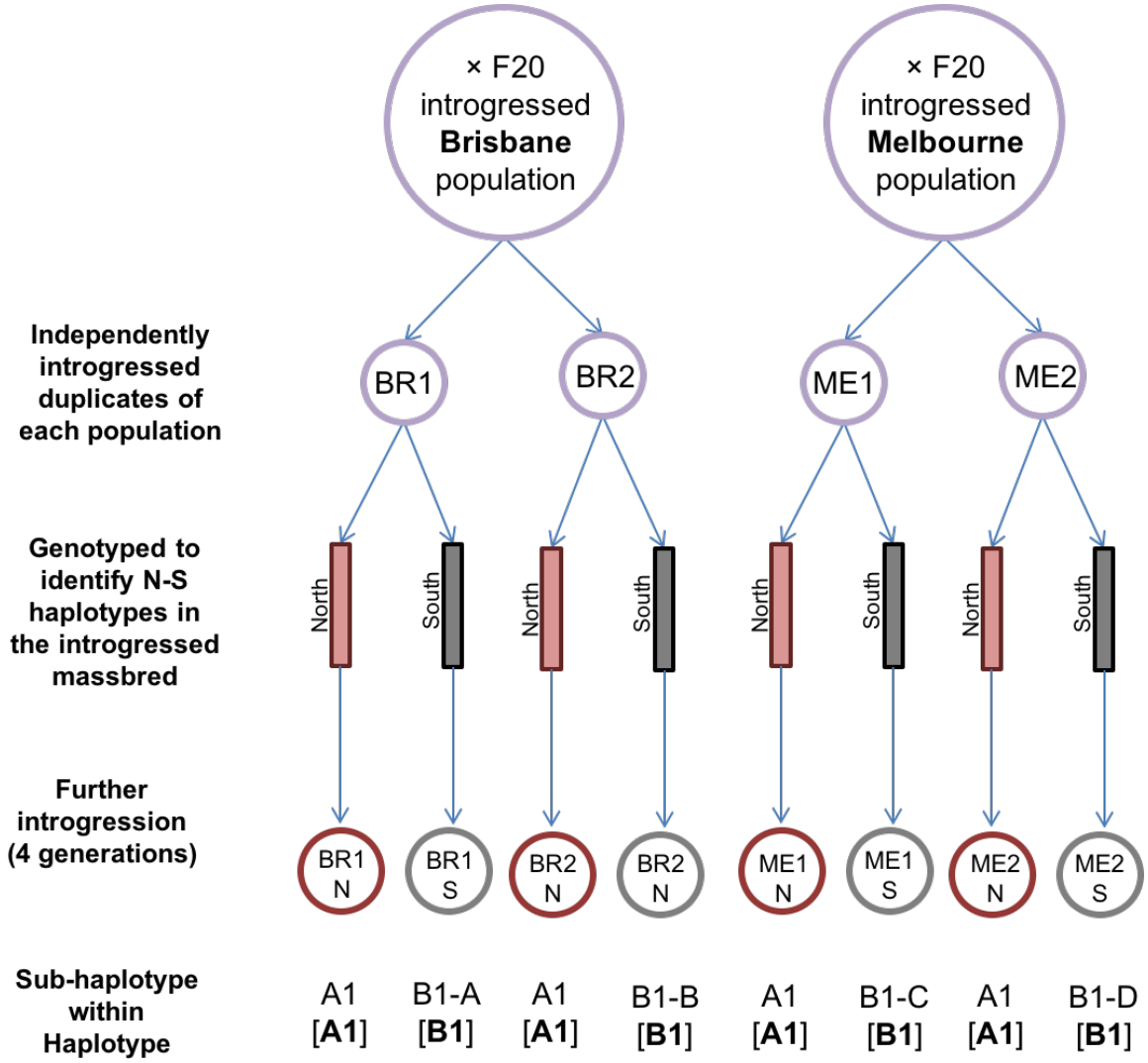

Figure S2B: Schematic of experimental setup (part 2). This figure illustrates the creation of eight new mitochondrial strains from the introgressed population duplicates (see Figure S2A). Following 20 generations of introgression into the Puerto Montt nuclear background, we sourced A1 and B1 haplotypes from each of the population duplicates of each of two introgressed populations; Melbourne (ME) and Brisbane (BR). To initiate each of these strains, one female with an A1 haplotype, and 1 with an B1 haplotype was taken per population duplicate and crossed to one male of PUE. One daughter of each of these crosses was then backcrossed to 1 PUE male, each generation for seven generations. Backcrossing was done in triplicate in case one of the crosses did not produce offspring. Next generation sequencing of these eight strains identified four unique sub-haplotypes within the main B1 haplotype, whereas the A1 haplotype was isogenic across all four strains.

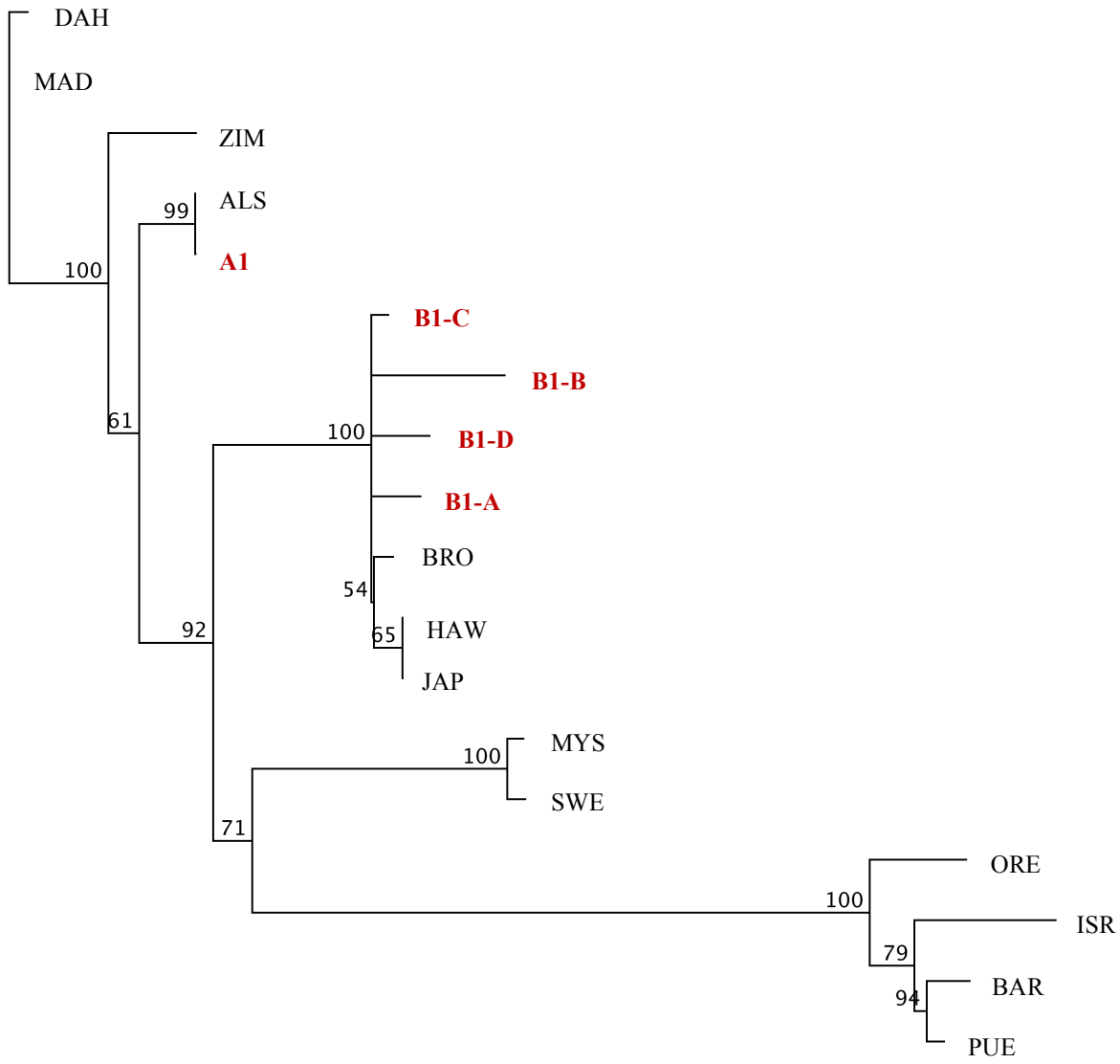

Figure S3: HKY neighbor-joining tree of 19 *Drosophila melanogaster* mitochondrial protein-coding regions. Protein-coding regions from the haplotypes obtained in this study are in red (A1, B1-A, B1-B, B1-C, B1-D), whereas the rest of the haplotypes were obtained from Camus et al. (2012) and represent haplotypes derived from worldwide locations: Alstonville, New South Wales, Australia (ALS); Barcelona, Spain (BAR); Brownsville, Texas, USA (BRO); Dahomey -now Benin- West Africa (DAH); Hawaii, USA (HAW); Israel (ISR); Japan (JAP); Madang, Papua New Guinea (MAD); Mysore, India (MYS); Oregon, USA (ORE); Puerto Montt, Chile (PUE); Sweden (SWE); and Zimbabwe (ZIM).